Microsc. Microanal. 18, 728–729, 2012
doi:10.1017/S1431927612001444

Microscopy AND Microanalysis

© MICROSCOPY SOCIETY OF AMERICA 2012

Introduction: Modern Imaging in Biology and Medicine: Papers from the Seventh Omaha Imaging Symposium, April 2011

The ability to see, or visualize, a phenomenon is an essential tool of modern biological research. Our ability to create static and dynamic images has grown exponentially in the 25 or so years since confocal microscopy became readily available. The ingenious and energetic application of insights from optical physics to biological imaging in recent years has brought us far-reaching extensions of simple imaging, including nonlinear (or multiphoton) excitation, total internal reflection imaging, and even single molecule counting techniques. The annual Omaha Imaging Symposium has since 2003 brought together experts in advanced biological imaging techniques for a one-day exposition of how these techniques help move biological science forward. The seventh iteration of the series was held Friday, April 8, 2011, at the Harper Student Life Center of Creighton University, in Omaha, Nebraska. This special section of *Microscopy and Micronanalysis* consists of papers from speakers at the symposium.

In multiphoton confocal microscopy, fluorophores are excited by the collision of multiple low-energy near-infrared photons. The use of near-infrared photons, which are less susceptible to scattering than visible wavelength photons, enables deeper penetration of tissue, while their smaller excitation volume reduces photoxicity. Thus multiphoton excitation is the preferred choice for deep imaging in living tissue. Two papers from the symposium highlight the advantages of multiphoton confocal microscopy. While multiphoton confocal imaging of brain and brain slices is becoming commonplace, investigators such as the McGavern laboratory at the National Institutes of Health are showing its utility for tracking immune cells in a variety of tissues, especially when the immune cells express fluorescent proteins as markers. The paper by Herz et al. (pp. 730–741) reviews developments in multiphoton confocal microscopy and provides a useful guide to its use in the study of immune cells. In a second paper, Dunaevsky (pp. 742–744) shows how time-lapse multiphoton confocal microscopy (correlated with electron microscopy) is being used to elucidate the critical roles of glia in synapse formation in the central nervous system.

Microscopists have long known that object resolution is ultimately limited by the diffractive properties of light. While confocal microscopy clarifies images by rejecting out-of-focus light, it ultimately does nothing to improve spatial resolution. Thus, microscopists have been excited to learn that several optical techniques have recently been developed to circumvent the diffraction limit, and at least one such instrument has become commercially available (from Leica). David Zenisek, from the Department of Cellular and Molecular Physiology at Yale University, studies molecules that regulate synapse formation and synaptic release in photoreceptors. The molecules are few in number and in photoreceptors are clustered at very small specializations called synaptic ribbons. The Zenisek laboratory paper (Lv et al., pp. 745–752) demonstrates the use of the Leica stimulated emission depletion method of superresolution imaging to show how one protein, RIBEYE, regulates the localization of calcium channels at the ribbon.

Confocal microscopy is not just for pretty pictures. As the paper from the Ricci laboratory at Stanford University demonstrates (Castellano-Munõz et al., pp. 753–760), the combination of spinning disk confocal microscopy and calcium-sensitive dyes is fast enough and simultaneously has the spatial resolution to enable the localization of the ionic flux through single (or small clusters of) mechanically-sensitive ion channel. These rapidly-activated channels are localized to fine microvillus-like projections on hair cells called stereocilia and are activated by pulling on even finer protein linkages between pairs of stereocilia. As the study shows convincingly, the channels are located on the taller

https://doi.org/10.1017/S1431927612001444 Published online by Cambridge University Press

stereocilia. The paper also describes the specialized apparatus required to synchronize mechanical stimulation and confocal image acquisition.

While many of the advances described in these papers rely on fluorescent labels, the next paper (Vergen et al., pp. 761–770) employs the natural fluorescence of cells. What we normally call background, and we struggle to reject, consists of the emissions of metabolites and proteins that are the normal constituents of cells. One particular naturally-occurring fluorophore is the reducing agent nicotinamide adenine dinucleotide (NADH), which is an essential intermediate in oxidative metabolism and is found abundantly in mitochondria and elsewhere. When oxidized (to NAD+), NAD loses its intrinsic fluorescence. Thus the levels of NADH fluorescence are a sensitive indicator of cellular metabolic state. This study, from the Nichols laboratory at Creighton University, takes the study of NADH in metabolism one step further by analyzing fluorescence lifetime images of cells under a variety of conditions. As the study indicates, measurement of NADH fluorescence lifetime, here performed by time-correlated single photon counting, reveals much new information about the metabolic state.

Ensemble methods of molecular behavior have well-understood disadvantages. Detailed studies of the kinetics of mechanisms such as folding and binding must be inferred from ensemble average behavior of many molecules. Improved detection methods, particularly electron-multiplying charge-coupled device cameras, have in recent years enabled the imaging and tracking of single fluorophores. Methods such as these have been exploited in a variety of ways. In the final paper, Hallworth and Nichols (pp. 771–780) discuss the application of single molecule imaging to a particular problem—the stoichiometry of membrane proteins. Traditional studies of membrane protein stoichiometry have relied on methods such as gels that remove the protein from its native environment. In the new approaches, single molecules of the proteins, with each subunit coupled to a fluorescent protein, are isolated in cell membranes or isolated membrane fragments. The molecules are exposed to continuous excitation sufficient to bleach the fluorescent proteins. By counting the number of step decrements of fluorescence observed until background levels are reached, the number of subunits in the molecule can be inferred. Several applications of this approach are discussed, and the technical advantages and limitations of each are highlighted.

The symposium speakers and audience agreed that the symposium was a stimulating and educational experience for all concerned. We hope you find the accompanying papers equally valuable.

Richard Hallworth
Co-editor

Michael G. Nichols
Co-editor

https://doi.org/10.1017/S1431927612001444 Published online by Cambridge University Press